

In vitro molluscicidal activity of wormwood (*Artemisia dubia*) leaves against *Oncomelania hupensis quadrasi*

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ABSTRACT

There have been many efforts to eradicate the problem of schistosomiasis. One method is to control the growing snail population. This study aimed to determine the molluscicidal activity of wormwood (*Artemisia dubia*) leaves methanolic extract against adult and juvenile *Oncomelania hupensis quadrasi*. Using infusion technique, 80% methanol was used as solvent. There were eight treatments used: distilled water (T0_(c1)) and 1% methanol (T0_(c2)) as negative controls; 0.0002% (2mg L⁻¹) niclosamide (T0_(c)) as positive control; and 11.121% (T1), 12.478% (T2), 14% (T3), 15.708% (T4) and 17.625% (T5), which were replicated five times with 10 snails per replicate.

Results showed that for adults, the wormwood leaves methanolic extract was not statistically significant with the commercially available molluscicide, although 17.625% (T5) resulted in 82% mortality rate. For juveniles, the different extract concentrations resulted in 98 to 100% mortality, which were comparable to 0.0002% (2mg L⁻¹) niclosamide and were considered highly effective based on Reik & Keitz (1954) and European Agency for the Evaluation of Medicinal Products (EMA). Thus, wormwood leaves methanolic extract has a promising molluscicidal activity against adult snails at a concentration of 17.625%, and at all concentrations for the juvenile snails of *O. hupensis quadrasi*. The LC₅₀ of the adult and juvenile snails were 14.075% and 10.294%, respectively.

Secondary metabolites that were found positive during the qualitative phytochemical analysis with the extract were tannins, saponins, and terpenoids. The mortality of the snails can be attributed to the bioactivity of the secondary metabolites present that may be acting in combination or individually.

Keywords: *Artemisia dubia*, wormwood, *Oncomelania hupensis quadrasi*, molluscicide

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INTRODUCTION

Synthetic molluscicides have major drawbacks on their efficacy (Michael et al 2013). This prompted the growing interest on investigating the potential of plants as alternatives. In schistosomiasis endemic areas, especially in poor countries, snail control is a challenge due to the very high cost of synthetic compounds. One of the methods in controlling schistosomiasis is to control the snail population with the use of molluscicides. Synthetic molluscicides cause stress on the water balance systems (McCullough et al 1980) and snails have evolved resistance (Duke et al 2010). This leads to increase of interest on plant molluscicides due to low cost (Adewunmi 1991).

Over the years, many researches have been conducted to look for a solution to control the snail population in the hopes of eradicating schistosomiasis through the use of plants (Hostettmann 1982, Ojewole & John 2004). In this study, wormwood (*Artemisia dubia*) plant was tested against *Oncomelania hupensis quadrasi*. Wormwood is an effective herbal medicine in treating a variety of disorders of the digestive tract. It is found to have antifungal, antibacterial, expectorative and antiasthmatic properties (Hoffman 2003). In addition, the plant is thought to be effective against a wide range of parasitic infections (Harris & Harris 2003).

MATERIAL AND METHODS

Preparation of Wormwood Leaves

Wormwood leaves regardless of maturity were collected within Baybay City, Leyte, Philippines and was further identified for correct classification; these were washed with tap water and air-dried at room temperature for 48h. After drying, the leaves were chopped and air-dried further for 7 days.

Plant Leaves Extraction

Plant extraction through methanol infusion for 48h was performed as previously described by Macabeo et al (2005). Methanol (80%) was used as it is an ideal solvent for plant active compounds extraction. However, it is more toxic than ethanol but is easily evaporated from the extract at low temperature (Zhao et al 2009). According to Azmir et al (2013), methanol is toxic if it is still present in the extract but is useful when extracting certain phytochemicals and unique compounds. After infusion, the collected filtrate was concentrated with a rotary evaporator (30°C).

Collection of *Oncomelania hupensis quadrasi*

Staff from the Medical Zoology Laboratory- Schistosomiasis Research and Training Center of Palo, Leyte, Philippines assisted the collection of *O. hupensis quadrasi* snails at Brgy. Guindag-an, Lumbia Swamp, Tanauan Leyte. Adult and juvenile snails were placed and stored in a glass jar layered with moistened filter paper; these were transferred to the Diagnostic Laboratory of the College of Veterinary Medicine of Visayas State University, Philippines.

Assay of Molluscicidal Activity

There were eight treatment groups. Each treatment group had a corresponding five replicates, with 10 adult or juvenile snails per replicate. Each replicate was flooded uniformly with varying concentrations of wormwood methanolic extract (WLME). The different treatment groups were: T0₍₋₎1 (distilled water), T0₍₋₎2 (1% methanol) as negative controls; T0₍₊₎ (0.0002% (2mg L⁻¹) of niclosamide) as positive control; 11.121% WLME (T1), 12.478% WLME (T2), 14% WLME (T3), 15.708% WLME (T4) and 17.625% WLME (T5). The different concentrations were based from the results of the lethal concentration that could kill 50% (LC₅₀). The LC₅₀ was established first using different concentrations of WLME (14%, 16%, 18% & 20%). Meanwhile, 50% mortality was observed at 14% while other concentrations resulted to more than 50% mortality. Then, using the logarithmic methods of Guevara & Recio (2005), the concentrations used in the final study were established.

Flooding technique was used in the assay, using only the live and viable snails. Juvenile and adult snails were sorted out in different petri plates for separate treatments. Juvenile snails measure 2.0-3.0mm long while adult snails measure 3.1-3.5mm long. They were exposed with the WLME for 24h. The plates were covered with cloth and tied with rubber bands. On the following day, the snails were transferred to another petri plate with distilled water and left for another 24h to further observe whether it is dead or alive (Rug & Ruppel 2001). Live snails were active after treatment, or when their head, belly-foot part and tentacles were extended as if attempting to crawl. To differentiate snails that were alive or dead, motility and sensitivity tests were conducted. For the motility test, the snails were transferred to another petri plate with distilled water layered with filter paper marked with 5mm diameter circles; those that moved out of the circles were considered alive while those that remained immobile and retained their positions after 3h were tested for sensitivity testing. For sensitivity testing, the snails were viewed with the use of a microscope (10X) and were prodded with a needle. When prodded, live snails usually retract their operculum; snails that were immobile but sensitive to prodding were considered alive.

Different formulas were used to determine the efficacy of WLME: 1) percent mortality and % efficacy based on Reik and Keitz (1954); wherein <70% mortality is ineffective, 71-80% mortality is effective and 81-100% mortality is said to be highly effective and 2) European Agency for the Evaluation of Medicinal Products (EMEA 2000); wherein <90% mortality is not effective.

Phytochemical Analysis of the Plant Leaves

Following the procedures as previously described by Senguttuvan, Paulsamy, Karthika (2014), qualitative phytochemical analysis of the WLME was conducted by testing the following secondary metabolites through different procedures and tests: alkaloids (Mayer's test, Wagner's test); tannins (Tannins test); saponins (Froth test); flavonoids (Alkaline Reagent test, Lead acetate test, Ferric Chloride test); and terpenoids (Chloroform & Sulfuric acid test). A grading system of the phytochemicals was described in the following manner: (+) positive, (-) traces and (0) undetected.

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Statistical Analysis and Experimental Design

The experiment was laid out in Completely Randomized Design (CRD). ANOVA was used to analyze the variation among groups and Tukey's HSD (honestly significant difference) test to determine comparison among groups.

RESULTS AND DISCUSSION

Variation in the number of dead adult snails was observed and dose-dependent (Table 1). The efficacy of the WLME according to percent mortality rate and its equivalent % efficacy based on Reik & Keitz (1954) were: for 17.625% WLME (T5), highest mortality rate of 82% which is highly effective; followed by 15.708% WLME (T4), with 74% mortality which is considered effective; and all the three remaining treatments 14% (T3), 12.478% (T2), 11.121% (T1) were considered not effective with mortality rates of 60%, 26% and 6%, respectively. However, based on to the EMEA, all treatments were considered not effective since their mortality rates were below 90%. Moreover, 17.625% WLME (T5) which had the highest mortality rate was not statistically comparable with 0.0002% (2mg L⁻¹) niclosamide, with 100% mortality rate. The LC₅₀ of WLME for adults was 14.075% based on Probit analysis at 95% confidence level.

Table 1. Mean % mortality of wormwood leaves crude methanolic extract against adult and juvenile *Oncomelania hupensis quadrasi* snails

Treatment	Adult Snails			Juvenile Snails		
	Average	Dead (Ave)	Mortality Rate (%)	Average	Dead (Ave)	Mortality Rate (%)
T0 ₍₋₎₁	10	0	0 ^a	10	0	0 ^a
T0 ₍₋₎₂	10	0	0 ^a	10	0	0 ^a
T0 ₍₊₎	10	10	100 ^e	10	10	100 ^b
T1	10	0.6	6 ^a	10	9.8	98 ^b
T2	10	2.6	26 ^b	10	10	100 ^b
T3	10	6	60 ^c	10	10	100 ^b
T4	10	7.4	74 ^d	10	10	100 ^b
T5	10	8.2	82 ^d	10	10	100 ^b

Means with different superscript letters are significantly different at $p < 0.05$

No. of replicates per treatment = 5

No. of snails per replicate = 10

For the juvenile snails, there was no variation in the level of mortality (Table 1). The efficacy of the WLME according to mortality rate and its equivalent % efficacy based on Reik and Keitz (1954) were: 11.121% WLME (T1) had the lowest mean mortality rate of 98%; 12.478% WLME (T2), 14% (T3), 15.708% (T4), and 17.625% WLME (T5) all had 100% mortality and were considered highly effective. Based on EMEA, all the treatments were also considered effective. All five treatments were also statistically comparable with the commercially available molluscicide. The LC₅₀ of the WLME for juveniles was 10.294% based on Probit analysis at 95% confidence level.

Phytochemical analysis was conducted in order to determine the possible secondary metabolites as bioactive components that might have contributed to the molluscicidal activity of the WLME. Based on the phytochemical tests conducted, tannins, terpenoids, and saponins were present. The positive and negative reactions were shown as qualitative interpretation of the results (Table 2). A blue-black and brownish green discoloration of the test solution indicated condensed tannins; terpenoids as reddish brown discoloration of the test solution; saponins, was indicated as copious lather formation. All tests for alkaloids and flavonoids were negative.

Table 2. Phytochemical analysis of plant secondary metabolites from wormwood leaves crude methanolic extract

Secondary metabolites	Qualitative grading
Alkaloids	0
Tannins	++
Terpenoids	+++
Flavonoids	0
Saponins	++

+++ strongly positive, ++ moderately positive, + weakly positive, - traces, 0 undetected Interpretation was based on the study of Senguttuvan et al (2014)

The WLME exhibited potential molluscicidal activity against adult and juvenile *O. hupensis quadrasi* snails at 24h exposure. The efficacy based on the mortality rates attained could be attributed to the secondary metabolites identified during the phytochemical test such as tannins, terpenoids, and saponins. These classes of compounds, independently or in combination, may be responsible for the mortality of snails. This finding conforms to the report of Aladesanmi (2007) in which similar constituents were found to exhibit molluscicidal activity.

Schultz (2018) and Smith, Zoetendal, and Mackie (2005) indicated that tannins bind to proteins and enzymes that may block digestion of an organisms. On the other hand, Aladesanmi (2007) and Hostettmann (1982) indicated that saponins exhibit strong molluscicidal properties. Moreover, Bak (2013) illustrated that saponins can disrupt cell membranes, cause cell death, and may ultimately kill herbivores and pests. For terpenoids, their molluscicidal activity could possibly be due to the action of thiol-containing enzymes. The action of terpenes or terpenoids most likely involves the alkylation(s) of the essential enzymes of the organism's metabolism (Knobloch et al 1989, McGarvey & Croteau 1995).

Although the level of bioactive compounds present in the plant depends on the season, other factors like the type of soil, temperature, altitude, radiation, atmosphere, light exposure, and nutrition can also influence the variability in plant metabolite levels that could affect the safety and the reliability of the plant's efficacy (Pavarini et al 2012).

On the variability between adult and juvenile mortality, Mohammed (2013) and Arufe (2004), explained that early life stages of snails are more sensitive compared to adults. Often, these were due to differences between life stages in the morphological, physiological, and even biochemical characteristics. Such factors include, but are not limited to 1) juvenile's organ systems may be sensitive to the

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effects of toxicants, and 2) differential rates of absorption or uptake distribution or detoxification (Aladesanmi 2007, Mohammed 2013). Also, toxicants may induce the synthesis of some specific proteins like oxygenase and methyllotheinins which may play a role in detoxifying and sequestering toxicants, thus rendering or reducing their toxic effect. Additionally, early life stages could be lacking fully-expressed enzyme systems needed for efficient detoxification and elimination of toxicants which could be due to the slow development of their organs (Anto et al 2005, Lardans & Dissous 1998).

CONCLUSION

Wormwood leaves methanolic crude extract has a potential molluscicidal activity, thus, can be used as an alternative molluscicides against *O. hupensis quadrasi*. Although the results of the efficacy against adult is not comparable to the commercially available molluscicide, the concentration rate can be increased to improve efficacy. Field trial of the extract is highly recommended.

COMPETING INTERESTS

The authors declare no competing interests.

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